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CHANGES IN ABUNDANCE OF IgG 2a mRNA IN THE NUCLEUS AND CYTOPLASM OF A MURINE B-LYMPHOMA BEFORE AND AFTER FUSION TO A MYELOMA CELL

CHRISTINE MILCAREK,* MAKIKO SUDA-HARTMAN and SUSAN C. CROLL

University of Pittsburgh, School of Medicine, Department of Molecular Genetics and Biochemistry, Pittsburgh, PA 15261, U.S.A.

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Abstract—Changes in IgG mRNA half-life, transcription and nuclear and cytoplasmic abundance were studied in two cell lines which contain an identical Ig γ 2a heavy chain but which differ in its expression. The A20.2J mouse lymphoma expresses about equal amounts of Ig γ 2a secretory- and membrane-specific mRNAs whereas in the AXJ hybrids, resulting from the fusion of A20.2J with the J558L myeloma, the secretory-specific form dominates. Further evidence of dominance of the myeloma phenotype was seen in the large changes in mRNA abundance and nuclear accumulation as well as in a small increase in Ig γ 2a mRNA half-lives for both secretory and membrane forms. Contributing to the observed >100-fold increase in the ratio of secretory vs membrane forms of the Ig γ 2a heavy chain in the AXJ hybrids are both a 10-fold decrease in the production of the membrane form by post-transcriptional RNA processing events and a ≈6–7-fold decrease in the nuclear to cytoplasmic ratio for the Ig secretory γ 2a and κ light chain RNAs. Differential RNA accumulation in the nucleus in the lymphoma cell therefore contributes to the differential expression of Ig secretory mRNA.

Key words: polyadenylation, mRNA, Ig mRNA, RNA transport.

INTRODUCTION

It is possible to produce at least two forms of mRNA and subsequently two forms of the protein product from a single, rearranged Ig heavy chain gene. These two forms are the secretory- and membrane-encoding mRNAs which serve as the templates for the secreted form of the protein or the antigen receptor on the membrane of B cells, respectively. Expression of these two forms of mRNA varies with the developmental stage of the B cell. In mature and memory B cells, and their transformed cell-type analogs, lymphoma cells, the production of the membrane-encoding form (mem) of Ig heavy chain predominates, whereas in plasma cells and their transformed analogs, the myeloma cell, secretory-encoding forms (sec) of Ig predominate, regardless of the heavy chain produced by that cell. B cell stage-specific expression of the two Ig mRNA forms could theoretically result from differential nuclear processing, transcription termination between the two polyA sites, cytoplasmic half-lives and/or nuclear accumulation. Nuclear accumulation of Ig heavy chain mRNA has until now remained an unexplored area.

In experiments using transfected, potentially alternatively spliced viral constructs, splicing seems to be unchanged in the various B cell stages (Peterson *et al.*, 1991). An increase in the use of the secretory-specific polyA site in plasma cells vs. the mature/memory B cells has been demonstrated for μ, α and γ genes (Lassman *et al.*, 1992; Milcarek and Hall, 1985; Peterson *et al.*, 1991; Seipelt and Peterson, 1995). Therefore increased nuclear polyadenylation appears to be a common mechanism for these three heavy chain classes.

The termination site for Ig transcription shifts in location during maturation to plasma cells in some Ig heavy chain genes but not in others. The region of transcription termination in the μ locus shifts from downstream of the membrane polyA site to a more 5′ location within the μ membrane exons following LPS stimulation of resting splenic B cells (Weiss *et al.*, 1989) and an inducible B cell line, BCL1 (Yuan and Dang, 1989). Meanwhile, in the α and γ genes no changes in the site of transcription termination have been observed in early vs late stage B cells (Flaspohler and Milcarek, 1990; Lebman *et al.*, 1992). The differences observed between the heavy chain classes with respect to shifts in termination site may reflect the differences in the relative strengths of the secretory specific polyA sites and the observation that

*Author to whom correspondence should be addressed.

Abbreviations: Ig, immunoglobulin; mem, membrane encoding form; polyA, polyadenylic acid; sec, secretory encoding form.

there is a coupling of transcription termination to polyA site strength (Edwalds-Gilbert *et al.*, 1993). In plasma cells, the μ sec polyA site seems to be stronger than the α sec site (Peterson *et al.*, 1991); this extra strength may, in combination with a DNA binding site in the CH4 to M1 exon of μ (Law *et al.*, 1987), enhance the more 5′ transcription termination in the μ gene in some very late stage of plasma cell development. The γ sec polyA site seems very weak and hence inefficient at causing transcription termination even when made stronger in plasma cells (Flaspohler *et al.*, 1995).

Heavy chain gene differences in message half-lives have also been observed. When message half-life was studied in cultured cells and splenic B cells, an increase in the stability of the μ mRNA in later stage/more mature cells was observed (Cox and Emtage, 1989; Jack and Wabl, 1988; Jack *et al.*, 1989; Mason *et al.*, 1988; Genovese and Milcarek, 1990). In one study, the half-lives of both the sec and mem forms of μ increase in stimulated cells, while the half-lives of both forms of δ heavy chain mRNA remain unchanged; this suggests that sequences within the body of the mRNA, i.e. μ CH1-4, influence stability (Reed *et al.*, 1994; Yuan and Dang, 1989). The μ and δ messages have identical translation rates (Yuan, 1984), so differences in the polysome content of activated vs resting cells cannot be the major determinant of μ stability as initially suggested (Jack *et al.*, 1989; Mason *et al.*, 1988). In contrast, others have reported that only the half-life of the sec μ mRNA increases in activated lymphocytes (Berberich and Schimpl, 1990). When an α producing derivative of the CH12 mouse B cell line was stimulated with LPS or IL-4 plus IL-5, the α sec but not the mem form of mRNA was stabilized, along with κ mRNA. Those authors suggested that the 107 nts at the 3′ end of α sec mRNA act as a target for potential trans acting factors that may play a role in stability (Eckmann *et al.*, 1994). Epsilon heavy chain mRNA shows a decrease in secreted but not membrane forms following FcεR-II stimulation of a human plasma cell line, suggesting differential stability of the two messages (Saxon *et al.*, 1991). Based on the observed differences in the other heavy chains we thought it would be informative to study γ sec and mem mRNA stability and processing. We wanted to control for differences in VH regions so we took advantage of the observation that fusion of a myeloma partner with the A20 gamma 2a producing lymphoma line, where sec = mem, resulted in increased levels of Ig secretion (Word and Kuehl, 1981), presumably because of increased levels of sec vs mem mRNA, although only protein levels and not RNA were determined in that study.

In our studies presented here, the plasma/myeloma cell phenotype is indeed dominant at the mRNA level in the fusion line, AXJ, which resulted from somatic cell hybridization between the γ 2a producing A20 lymphoma (sec = mem) and the J558L plasma/myeloma line, which when transfected with the gamma gene produces sec mRNA in great excess over the mem forms. We show that the half-lives of both the Ig sec and mem γ 2a species increase about 2-fold in the AXJ fusion cells relative to A20 cells. Therefore in this system differential changes in γ 2a heavy chain RNA half-life do not account for differential cytoplasmic mRNA levels of sec vs mem species. We show that γ 2a heavy chain transcription is not effected by the fusion. The ratios of sec to mem forms of Ig heavy chain in the nucleus and cytoplasm of the AXJ hybrids are like those of a γ 2a producing myeloma. We find that the nuclear vs cytoplasmic distribution of the sec form of the Ig RNA differs about 8-fold between the lymphoma and myeloma cell types, with the AXJ fusion resembling that of a plasma/myeloma. Our results suggest, for the first time, that nuclear transport of mRNA in the hybridoma cells appears to be different from that in lymphoma cells.

MATERIALS AND METHODS

Cell lines

Cells were grown in IMDM plus 5% heat inactivated horse serum, 10 u/ml penicillin and streptomycin (Gibco Laboratories) at 37°C in 5% CO_2. HAT medium is IMDM supplemented with: hypoxanthine at 10^{-4}M; aminopterin at 10^{-5}M; and thymidine at 3×10^{-5}M. Ouabain was used at 1.8 mM. The A20.2J mouse lymphoma cell line was obtained from Dr Mike Kuehl. The cells produce about equal quantities of secretory and membrane encoding IgG γ 2a heavy chain mRNA and a κ light chain. They were tested and found to be HAT sensitive and ouabain sensitive. J558L mouse plasma/myeloma cells were obtained from Dr Sherie Morrison. They are λ light chain producers, have lost their endogenous α heavy chain, but, behave as a myeloma when transfected with an IgG gene, i.e. sec > > mem mRNA production (Kobrin *et al.*, 1986). J558L cells were found to grow on HAT containing medium and fail to grow on 1.8 mM ouabain containing medium. K23 is a γ 2a, κ producing myeloma line which arose from a sister chromatid exchange from the γ 2b producing myeloma 4T001 (Morrison, 1979). K46P is a γ 2a, κ lymphoma (Kim *et al.*, 1979) obtained from Dr John Cambier. RPC5.4 is a myeloma line which is γ 2a, κ positive; we obtained it from the ATCC.

Ouabain selection

To each of two 80 cm^2 flasks, 2.4×10^6 A20.2J cells were added and allowed to adhere. The normal growth medium was removed and the adherent cells were fed with 20 ml medium (IMDM, 5% horse serum, plus penicillin and streptomycin) supplemented with 0.8 mM ouabain. Two days later fresh medium with ouabain increased to 1.1 mM was added; then 3 days later fresh medium with ouabain increased to 1.8 mM was added. Finally, two days later fresh medium with ouabain increased to 2.1 mM was added. At this time most of the cells had detached from the plastic and appeared to be dead as judged by trypan blue inclusion. Twelve days later, one small colony of cells was visible on one of the flasks. Cells from the colony were cloned by limiting dilution using conditioned medium plus ouabain in a 96-

well microtiter plate; the cells from the five positive wells were grown up and tested for continued HAT sensitivity and IgG expression, see below.

Cell fusion

One A20.2J Ou^R HAT^S clone, B5, with sec=mem γ 2a mRNA, was fused with the HAT^R Ou^S J558L line by polyethylene glycol fusion (Harlow and Lane, 1988). Hybrid cells were selected in medium containing one-half the previously indicated concentrations of HAT and ouabain. Six positive wells were subcloned by limiting dilution in conditioned medium. These six clones of fused/hybrid cells (now called AXJ) were tested for IgG mRNA and transcription, see Results.

RNA analyses

Nuclear and cytoplasmic RNAs were isolated as previously described (Kobrin *et al.*, 1986). Northern blots and nuclear transcription run-on experiments were performed essentially as previously described (Genovese and Milcarek, 1990). Single stranded riboprobes were used as the targets on nitrocellulose for the nuclear run-on experiments as previously described (Flaspohler and Milcarek, 1990; Flaspohler and Milcarek, 1992).

Half-lives were determined using decay of mRNA in DRB (5,6-dichloro-1-beta-ribofuranosyl benzimidazole) at 21 μm/ml (65 μM) as previously described (Genovese and Milcarek, 1990; Harrold *et al.*, 1991). DRB blocks new RNA synthesis in mouse myeloma and lymphoma cells, data not shown. Total cytoplasmic RNA was isolated from cells at 1, 2, 3, 4, 6, and 8 hr post DRB. Decay was monitored by quantitative Northern blot analyses in which equal A_{260} amounts were loaded and run on formaldehyde-containing agarose gels and transferred to nytran (Schleicher and Schuell) using 20 mM sodium phosphate pH 6.5. Blots were probed sequentially with the indicated [^{32}P]-labelled antisense RNA or nick translated double-stranded DNA probes with intermediate washings to remove the previous probe; quantitation was done either by scanning exposed X-ray film with a densitometer or by exposing the nytran filter to a screen which was then read in the phosphoimager. DRB does not influence cell viability or ribosomal RNA turnover as we have previously described (Genovese and Milcarek, 1990; Harrold *et al.*, 1991). Therefore, 18S ribosomal RNA was used as a loading control in the analyses.

Hybridization probes

The indicated genes were cloned into pGEM vectors (Promega) to make antisense probes for Northern hybridization or as immobilized probes for the run-on experiments: plasmid p2aCH3 includes the 312 bp SacI fragment of the Ig γ 2a gene covering the CH3 exon. Plasmid pCH3-TM encompasses the 1.3 kb region from the PstI site between CH2 and CH3 to the SmaI site upstream of M1. Plasmid pM1M2 includes the 1.15 kb KpnI to PstI fragment of the Ig γ 2a gene covering the M1 and M2 exons and a small portion of the membrane 3' untranslated region. Plasmid pGAPDH includes a 559 bp PstI to BalI fragment of the rat glyceroladehyde phosphate dehydrogenase gene. Plasmid pAL includes a 1.1 kb PstI fragment of the mouse beta-actin cDNA. These have been described previously (Flaspohler and Milcarek, 1990; Genovese and Milcarek, 1990).

The cDNAs for mouse Ig κ light chain (Schibler *et al.*, 1978), transcription factor pSII-3 (Hirashima *et al.*, 1988), dihyrofolate reductase, DHFR (Hook and Kellems, 1988), and cyclophilin (Friedman and Weissman, 1991) were nick translated using α-[^{32}P]dCTP and DNA polymerase I of *E. coli*. These were subsequently used for the Northern blots and half-life determinations.

Analysis of protein

Secretion of Ig γ 2a from the cells was measured by a Western blotting technique modified from Harlow and Lane (1988). Briefly, 10^6 rapidly growing cells were washed once and transferred to 2 ml serum free optimem medium (Gibco Laboratories) and allowed to incubate overnight at 37°C. The cells were harvested at 2000 g. The cell supernatant was then removed and spun again, at 7000 g, to remove any additional debris. Samples of 20 μL cell supernatant in 1 × sample/cracking buffer were boiled immediately before running on an SDS, 10% acrylamide gel in 0.4 M Tris pH 8.8 with a 0.5 M Tris pH 6.8 stacking gel of 6.8% acrylamide, run with Tris-glycine buffer (Harlow and Lane, 1988). Kaleidoscope markers (Biorad) were run as size controls. The gel was then blotted to PolyScreen/PVDF membranes (NEN Research Products) and probed with alkaline-phosphatase conjugated rabbit anti-mouse Ig serum (Sigma) and developed with BCIP/NBT (Sigma) as described (Harlow and Lane, 1988).

RESULTS

AXJ fusion cells behave like myeloma/plasma cells

Changes in IgG mRNA abundance and half-life were studied in two cell lines that contain an identical Ig heavy chain but differ in its expression. The memory B cell line, A20.2J (Ig γ 2a, κ), has equivalent levels of Ig gamma secretory-encoding (sec) and membrane specific (mem) mRNAs. Prior to fusion, ouabain resistant subclones of A20.2J were selected and characterized for sec and mem specific Ig γ 2a mRNA expression by Northern blot. Four out of five ouabain resistant subclones continued to produce IgG heavy chain mRNA like the parent A20.2J line. The secretory to membrane encoding mRNA levels in these was ≈1:1, data not shown.

One A20.2J HAT^S Ou^R subclone, B5, was fused to the J558L HAT^R Ou^S line and clones which were simultaneously HAT and ouabain resistant were selected. J558L is a cell line which lacks endogenous heavy chain production but when transfected with an IgG gene is capable of producing primarily sec specific IgG mRNA (Kobrin *et al.*, 1986). Northern blots were performed on six independent clones which resulted from the fusion, see Fig. 1. In all six AXJ fusion clones, production of the

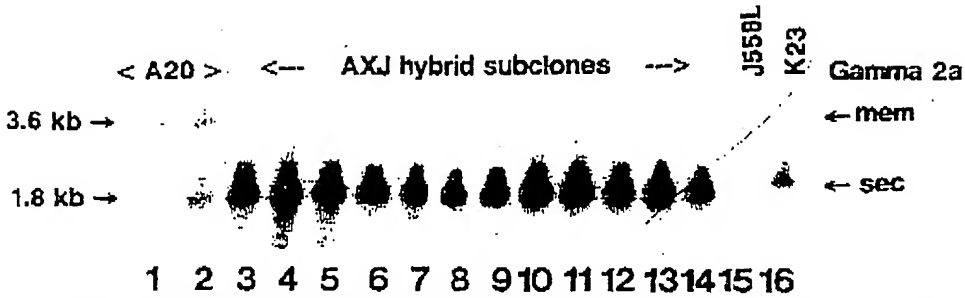

Fig. 1. Northern analysis of Ig γ 2a heavy chain mRNA from A20 and the AXJ hybrids. Cytoplasmic RNA (1 μg) from the indicated cells was run on a denaturing gel and blotted to nytran membranes. The RNA was bound to the nytran by UV-cross linking and then probed with a [^{32}P]-U-labelled, antisense p2aCH3 RNA which is specific for the CH3 constant region of the Ig γ 2a mRNA and detects both secretory (sec = 1.8 kb) and membrane-specific Ig mRNA (mem = 3.6 kb). 18 and 28S rRNA were used as size markers. Lanes 1–2, A20.2J Ou^R clone, B5. Lanes 3–14, Ou^{R}HAT^R AXJ hybrids; lanes 3–4, subclone I; lanes 5–6, subclone II; lanes 7–8, subclone III; lanes 9–10, subclone IV; lanes 11–12, subclone V; lanes 13–14, subclone VI. Lane 15, J558L. Lane 16, K23 γ 2a myeloma.

secretory-specific form of Ig heavy chain mRNA predominated over that of the membrane-encoding form, by ≈ 100:1. All the subclones continued to express the J558L parental λ Ig light chain mRNA, not shown, and the A20 parental κ Ig light chain, data shown below.

Secretion of the intact IgG from the cells was measured by Western blotting of the cell supernatant using a rabbit anti-mouse antibody, coupled to alkaline phosphatase. As shown in Fig. 2, neither the parental A20 nor the parental J558L cells secrete detectable amounts of IgG, whereas the AXJ hybrid fusion cells secrete heavy chain at levels comparable to that of an IgG2a producing myeloma, K23. Therefore, the ability to secrete the heavy chain is a property bestowed by the myeloma partner on the A20 endogenous H chain. The difference in the migration of the AXJ IgG2a heavy chain and that of the K23 heavy chain may be due to the fact that the two cells have different V-regions and may be subject to differential modifications. Secretion of the heavy chain protein is therefore dominant in the hybrids and is accompanied by the production of increased amounts of the sec mRNA.

Increased γ 2a secretory to membrane-encoding RNA ratios in the AXJ hybrids could be accounted for by: differential transcription termination prior to the membrane exons; an increase in the mRNA half-life of the secretory form relative to the membrane form; differential processing of the primary nuclear transcript (polyadenylation and/or splicing) to produce more of the secretory form; or by an increase in the export of the secretory form from the nucleus relative to the export of the membrane-encoding form. In addition, a combination of these mechanisms may be operative.

Ig heavy chain transcription is unaffected in the AXJ hybrids

Ig gene transcription rates were measured by run-on transcription experiments in isolated nuclei. In this assay, nuclei are incubated briefly with α[^{32}P]UTP and the incorporation of label into specific growing RNA chains is determined by hybridization to a selected set of immobilized, single stranded, anti-sense RNAs. Initiation of new RNAs is negligible and chain elongation is only a few hundred nucleotides, so that the assay effectively measures relative RNA polymerase loading on the various portions of the gene. Run-on transcripts from each cell line were hybridized to duplicate or triplicate slots of antisense RNA, locations shown in Fig. 3A, and processed as described previously. A representative autoradiograph from one such experiment is shown in Fig. 3B. Films from at least two separate experiments were scanned and the calculated transcription rates relative to

1 2 3 4 5 6 7

Fig. 2. Western blot of cell secretions from A20 and AXJ hybrids. Cells were incubated overnight in serum free medium. The secreted Ig in the cell supernatants was run on SDS–PAGE, blotted to a PVDF membrane and probed with alkaline-phosphate conjugated rabbit anti-mouse Ig and developed with BCIP/NBT. Lane 1, K46P γ 2a lymphoma; lane 2, A20.2J Ou^R B5; lane 3, AXJ subclone I; lane 4, AXJ subclone III; lane 5, J558L; lane 6, K23 γ 2a myeloma; lane 7, AXJ subclone V. Protein molecular weight size markers were run in a separate lane.

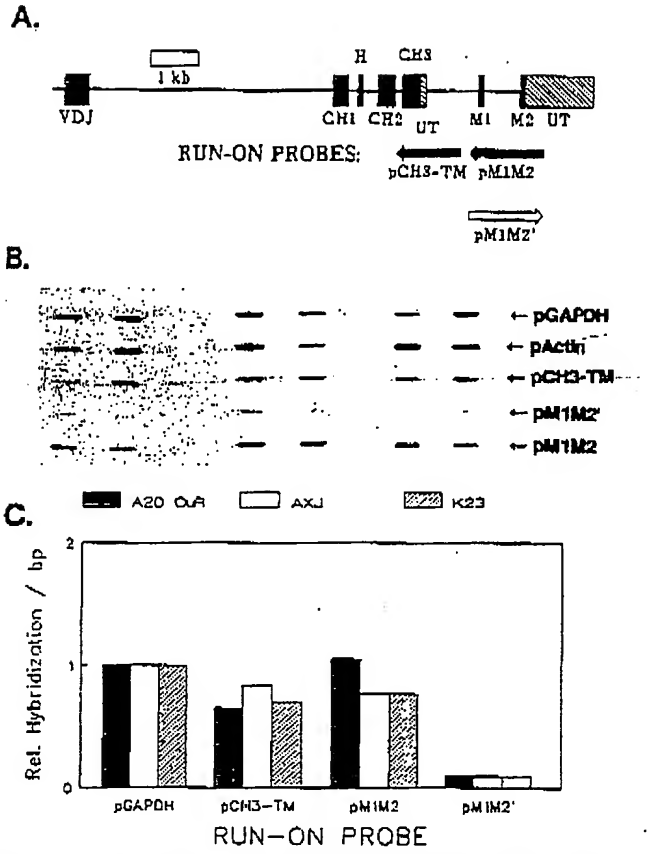

Fig. 3. Transcription run-on analyses of A20 and AXJ hybrids. Panel A, map of the mouse Ig γ 2a gene with the location of sequences used as run-on probes indicated below the map. The solid arrows indicate the locations of the antisense probe RNAs pCH3-TM and pM1M2; the open arrow indicates the sense strand probe pM1M2′ which should not hybridize with the labelled sense transcript from the Ig γ 2a gene. Panel B, nuclei from the indicated cells were labelled with [^{32}P] UTP. The RNA from those nuclei was hybridized with a filter on which single stranded RNAs were immobilized. pGAPDH contains antisense sequences for glyceroladehyde phosphate dehydrogenase. pActin contains antisense sequences for β actin. The location of the other probes is shown in panel A. Solid bar, A20.2J Ou^R B5 cells; open bar, AXJ hybrid III; striped bar, K23 γ 2a myeloma. Panel C, the relative hybridization per base to each run-on probe was calculated from several experiments. Hybridization intensities were normalized to 1.0 for the pGAPDH probe. Data for pActin is not shown in panel C. Solid bars, A20.2J Ou^R B5 cells; open bars, AXJ hybrid III; striped bars, K23 γ 2a myeloma.

the GAPDH internal control are shown in Fig. 3C. We conclude that there is no significant increase in the transcription of the γ 2a gene in the AXJ hybrids relative to that seen in the A20 parent, nor is there significant transcription termination occurring prior to the membrane exons. Therefore, transcription rate and termination point movement play little or no role in the increased production of secretory-specific forms of the heavy chain mRNA in these hybrids.

Cytoplasmic mRNA ratios in the AXJ hybrid cells resemble the myeloma pattern

In order to determine the absolute amount of the Ig γ 2a heavy chain mRNA in the cytoplasm and the nucleus of the AXJ hybrid cells, RNA was blotted to a membrane and then hybridized sequentially with probes for γ 2a, and GAPDH and 18S rRNA; cytoplasmic GAPDH mRNA and 18S rRNA have previously been shown not to vary between different B cell lines (Genovese and Milcarek, 1990; Harrold *et al.*, 1991). From the data in Fig. 4 and other similar blots (not shown), RNA abundancies were determined by using the amount of 18S ribosomal as a loading standard; the γ 2a membrane-encoding cytoplasmic RNA in A20.2J was set as 1.0 and all species were normalized to that value. The information about the GAPDH mRNA is summarized and discussed in a later section.

As summarized in Table 1, the overall amount of the cytoplasmic γ 2a heavy chain mRNA is increased 9.4-fold in the AXJ hybrids relative to the A20 parent. Therefore, not only is the ratio of secretory to membrane forms of the γ 2a mRNA altered by the hybridization to the myeloma, but the overall amount of the Ig heavy chain mRNA is also increased so that the AXJ hybrid appears "myeloma-like" in phenotype. In other words, the myeloma mRNA abundance phenotype is the dominant one in these hybrids.

The increase in the total cytoplasmic γ 2a mRNA levels in the hybrids could be accounted for by an increase in mRNA half-life, an increase in processing rates of the primary transcript, an increase in nuclear transit times, or a combination of these mechanisms, each at a smaller fold-increase. The >100-fold increased γ 2a secretory to membrane RNA ratios (93 vs 0.7, Table 1) in the AXJ hybrids could be accounted for by any of these processes acting differentially on the secretory encoding but not the membrane encoding form of Ig γ 2a mRNA.

Increased nuclear accumulation of γ 2a sec Ig mRNA in A20 cells contributes to decreased cytoplasmic levels

To investigate nuclear processing and accumulation, we compared the relative amounts of Ig γ 2a RNA found in the nucleus to that found in the cytoplasm from data like that shown in Fig. 4; the results are summarized in Table 1. Note that five times as much A_{260} amount of nuclear RNA as cytoplasmic RNA was loaded in the gel in Fig. 4, but the amount of Ig RNAs were normalized to 18S rRNA. A striking feature of this data is that the nuclear to cytoplasmic ratios in the two cell types differ substantially for the sec Ig RNA. The amount of secretory encoding RNA in the nucleus of the A20 cells (0.56 units) slightly exceeds that in the cytoplasm (0.4 units) for a nuclear to cytoplasmic ratio of 1.4; meanwhile, in the AXJ cells, the secretory-encoding nuclear RNA (1.1 units) is almost 9-fold less than that seen in the cytoplasm (9.3 units) for a nuclear to cytoplasmic ratio of 0.12. That is, the A20 cells seem less efficient at getting the secretory form of the Ig heavy chain mRNA out of the nucleus; alternatively the AXJ cells are more efficient

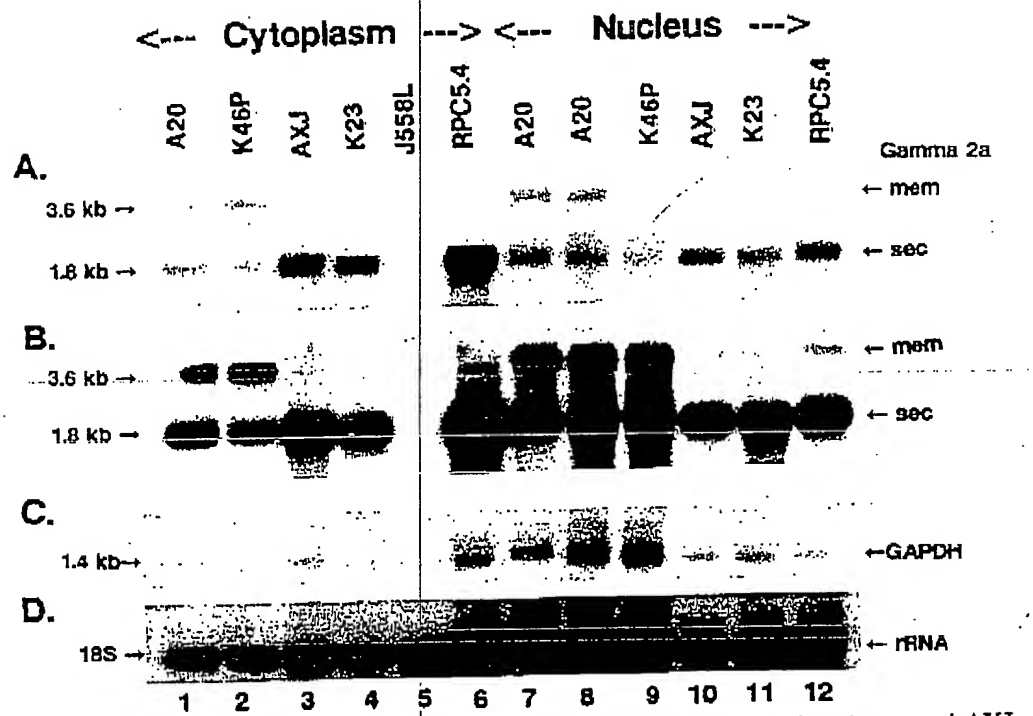

Fig. 4. Northern analysis of cytoplasmic and nuclear fractions of mycloma, lymphoma and AXJ hybrids. One microgram of cytoplasmic RNA or 5 μg of nuclear RNA (determined by A_{260}) from the indicated cells was run on a denaturing gel and blotted to nytran membranes. The RNA was bound to the nytran by UV-cross linking and then probed sequentially with [^{32}P] U-labelled antisense probes. Panels A and B, probed with p2aCH3, which is specific for the CH3 portion of Ig γ 2a detects both secretory (sec = 1.8 kb) and membrane-specific Ig mRNA (mem = 3.6 kb). In panel A the film was exposed to the blot for 18 hr; in panel B the exposure was 4 days. Panel C, probed with pGAPDH, glycerolaldehyde phosphate dehydrogenase. Panel D, probed with rRNA, specific for 18S ribosomal RNA. The previous probe was stripped from the nytran between each hybridization. Density of the rRNA signal was determined and used as a loading and transfer control and for normalization between samples. The ethidium staining of 18 and 28S rRNA was used as size markers. Lanes 1–6, RNA from the cytoplasmic fraction; lanes 7–12, RNA from the nuclear fraction. Lanes 1, 7 and 8, A20.2J Ou^R B5; lanes 2 and 9, K46P γ 2a lymphoma; lanes 3 and 10, AXJ hybrid III; lanes 4 and 11, K23 γ 2a myeloma; lane 5, J558L; lanes 6 and 12, RPC5.4 γ 2a myeloma.

at exporting the sec Ig mRNA. We also compared another γ 2a, κ producing lymphoma line, K46P, with a γ 2a, κ producing myeloma, RPC5.4, in Fig. 4 and saw an increased accumulation of the secretory form of the γ 2a mRNA in the nucleus of the lymphoma, consistent with a generalization of our observations to other cell lines. The nuclear to cytoplasmic ratio for membrane-encoding Ig RNAs are more difficult to calculate because the numbers are very small in the myeloma cells, and hence more subject to error.

We wanted to see if this effect on nuclear accumulation that we saw with the γ 2a secretory-encoding mRNA extended to other mRNAs, so we examined the distribution between the nucleus and cytoplasm for the RNAs of several genes as shown in Fig. 5 and summarized in Table 2. For κ Ig light chain and β-actin there

Table 1. Comparison of nuclear vs. cytoplasmic Ig γ 2a RNA abundancies[a]

RNA:	Cytoplasmic RNA A20	AXJ	K23	Nuclear RNA A20	AXJ	K23	Nuclear to cytoplasmic ratio A20	AXJ	K23
γ2a sec	0.4	9.3	8.3	0.56	1.1	1.34	1.4	0.12	0.16
γ2a mem	0.6	0.1	0.1	0.28	0.03	0.03	0.47	0.3	0.33
sec + mem	1.0	9.4	8.4	0.84	1.13	1.37	0.84	0.12	0.16
sec/mem	0.7	93	83	2.0	37	45	–	–	–

[a]Densitometry units, normalized for 18S ribosomal RNA in the same lane with the value of the γ 2a secretory (sec) plus membrane-encoding (mem) cytoplasmic mRNA species set to 1.0. Blots were exposed in the linear response range of the film for each of the species. Results are the averages of several determinations.

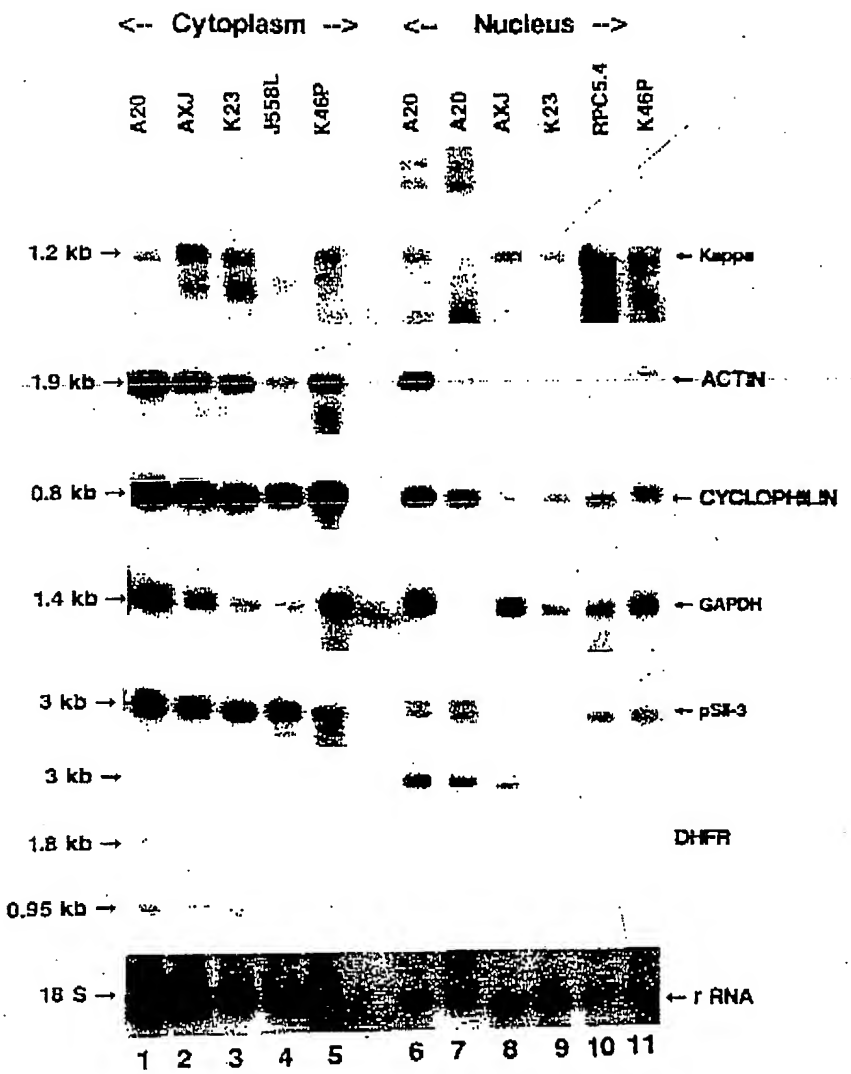

Fig. 5. Northern analysis of several mRNAs in the cytoplasm and nucleus of mycloma, lymphoma and AXJ cells. Five micrograms per lane of each RNA (determined by A_{260}) was run from the indicated cells, blotted to a nytran membrane and probed sequentially with riboprobes for: κ light chain, β actin (Actin), cyclophilin, glycerolaldehyde phosphate dehydrogenase (GAPDH), transcription elongation factor SII (pSII-3), dihydrofolate reductase (DHFR), and 18S ribosomal RNA (rRNA). The previous probe was stripped from the nytran between each hybridization. The ethidium staining of 18 and 28S rRNA was used as size markers. Lanes 1–5, RNA from the cytoplasmic fraction; lanes 6–11, RNA from the nuclear fraction. Lanes 1, 6 and 7, A20.2J Ou^R B5; lanes 2 and 8, AXJ hybrid III; lanes 3 and 9, K23 γ 2a myeloma; lane 4, J558L; lanes 5 and 11, K46P γ 2a lymphoma; lane 10, RPC5.4 γ 2a mycloma. Note that in lane 7, a bubble in the blotting prevented transfer of species in the 1.0 to 1.4 kb region. This lane was not used for any of the quantitations. DHFR mRNAs in the cytoplasmic fraction include 0.95 to 3 kb species in A20 and K46P cells, whereas in the nucleus only the 3.0 kb species was observed in some of the lanes.

is a significant increase in the nuclear to cytoplasmic ratio in the A20 cells over that seen in the AXJ fusion and K23 cells. RNAs for cyclophilin, an intracellular receptor for cyclosporin, GAPDH, and the transcription elongation factor SII, also show a small (≈2-fold) increase in the nuclear to cytoplasmic ratio in the A20 cells, relative to that seen in AXJ hybrids. The Ig γ 2a secretory-encoding form, κ light chain, and β-actin stand out having the biggest changes, whereas the Ig γ mem RNA seems to follow the cyclophilin, GAPDH, SII pattern.

Note in Figs 4 and 5 that the relative RNA levels in the nucleus and not the cytoplasm for the indicated mRNAs are what change between the cell types. We therefore conclude that the A20 cells differ from the AXJ in some aspect of general nuclear RNA metabolism; perhaps they are not transporting all of the tested RNAs as efficiently to the cytoplasm.

In addition to the 1.2 kb full length κ light chain RNA one can see a number of larger sized species of light chain RNA in the nucleus of the A20 cells, lane 6, Fig. 5, which are of diminished abundance in the AXJ and K23 cells: these larger species may reflect an overall slower post-

Table 2. Comparison of nuclear vs cytoplasmic abundancies for several RNAs[a]

RNA:	Nuclear to cytoplasmic ratio in: A20	AXJ
κ Ig light chain	3.0	0.5
β Actin	1.5	0.4
Cyclophilin	0.8	0.4
GAPDH	1.5	0.7
SII	0.4	0.2

[a]Ratio of nuclear to cytoplasmic densitometry units, normalized for 18S ribosomal RNA in the same blot. Blots were exposed in the linear response range of the film. Results are the averages of several determinations.

transcription processing rate of κ mRNA in the A20 cells. The DHFR gene is unusual in that there are seven tandem polyadenylation sites through which transcription proceeds unabated, producing many cytoplasmic species (Hook and Kellems, 1988). We see an accumulation of a 3 kb species in the nucleus whereas in the cytoplasm of the A20 cells there are three major species visible, the 0.95, 1.8 and 3 kb forms; meanwhile, in the cytoplasm of the AXJ and K23 cells only the 0.95 kb band is seen. Again, it appears that post-transcriptional RNA processing and nuclear export events in the A20 and K46P lymphoma cells differ from those seen in the AXJ hybrids, K23, and RPC5.4 myeloma cells. Because of the discrepancy in the observed nuclear vs cytoplasmic species for the DHFR gene, we did not calculate nuclear to cytoplasmic ratios for DHFR in Table 2; however these results are presented to show that the nuclear fractions are not significantly contaminated by the cytoplasmic contents.

Note that in lane 7 of Fig. 5, a duplicate A20 nuclear sample, there is a small region between 1.0 and 1.4 kb where poor blotting was observed; compare lane 7 with lane 6 where the GAPDH and other species in this size range were successfully transferred. Lane 7 was not used for quantiations in Table 2.

Changes in mRNA half-life do not account for the altered sec:mem RNA ratios in the AXJ hybrids

The stability of the γ 2a Ig mRNA was determined by treating the cells with DRB (5,6-dichloro-1-beta-ribofuranosyl benzimidazole) and assaying the amount of specific message, secretory-specific or membrane-encoding, remaining over time (up to 8 hr) using Northern hybridization or slot blots (Fig. 6). DRB blocks new polymerase II RNA synthesis while allowing decay of pre-existing cytoplasmic mRNAs, but not ribosomal RNA (Harrold *et al.*, 1991; Genovese and Milcarek, 1990). The amount of 18S ribosomal RNA was used as a loading control since we have previously shown that it is unaffected by DRB treatment. The stability of the GAPDH, SII-3 transcription factor, DHFR (species labelled 0.95 kb in Fig. 5) and κ light chain mRNAs were also determined (Fig. 6). Data for all the species tested are compiled in Table 3. The half-lives of the GAPDH, DHFR and SII mRNAs were the same in both cell types. For both sec and mem γ 2a Ig heavy chain mRNA, there is a small but significant increase in the half-life in the AXJ hybrids relative to that seen in the A20 parental line; κ mRNA shows a similar trend.

DISCUSSION

Dominance of plasma/myeloma cell phenotype in AXJ hybrids

Changes in IgG mRNA abundance, half-life and nuclear accumulation were studied in two cell lines which contain an identical Ig γ 2a heavy chain but which differ in its expression. The A20.2J γ 2a lymphoma expresses about equal amounts of Ig γ 2a sec and mem mRNA whereas the AXJ hybrids resulting from the fusion of A20.2J with the J558L myeloma express a large excess of the sec-form of Ig γ 2a mRNA. This myeloma-dominant phenotype was seen in all the six hybrid clones that we tested. Dominance of the myeloma phenotype was seen in large changes in mRNA abundance and nuclear accumulation as well as in a small increase in Ig γ 2a mRNA half-lives. Somatic cell fusions between fibroblasts and myelomas, or T cells and myelomas, result in the extinction of IgH gene expression at the level of transcription targeted to the Ig enhancer (Eckhardt, 1992; Greenberg *et al.*, 1987; Zaller *et al.*, 1988). Because transcriptional activation does not vary much between cultured B cell tumor lines representing various stages (Kelley and Perry, 1986) there is less likely to be extinction at that level in a lymphoma × myeloma hybrid. Our results with the AXJ hybrids indicate that post-transcriptional RNA processing events and not transcription of the IgG locus play a major role in the switch from sec to mem forms of Ig production.

Recent transfection and biochemical studies have suggested that there is a negative regulator of Ig sec polyA site use in lymphoma cells (Peterson, 1994; Yan *et al.*, 1995). One action of this negative regulator may be to cause the retarded exit of sec mRNA from the nucleus as we observe here. Whatever component(s) the myeloma contributes to the AXJ hybrid appears to overcome this negative regulator either by introducing a dominant positively acting factor, by repressing the negative regulator, or both.

Factors contributing to changes in sec:mem ratios

Our results indicate that a series of events contribute to the observed > 100-fold increase in the ratio of sec vs mem forms of the Ig γ 2a heavy chain in the AXJ hybrids when compared to the A20.2J parent; there are two major changes whose net effect is multiplicative. The first is a 10-fold decrease in the production of the membrane form of the γ 2a in the AXJ hybrids by post-transcriptional RNA processing events whereas the overall amounts of nuclear RNA remain relatively constant between the two cells. Differences in the polyadenylation patterns in lymphoma vs myeloma cells have previously been observed

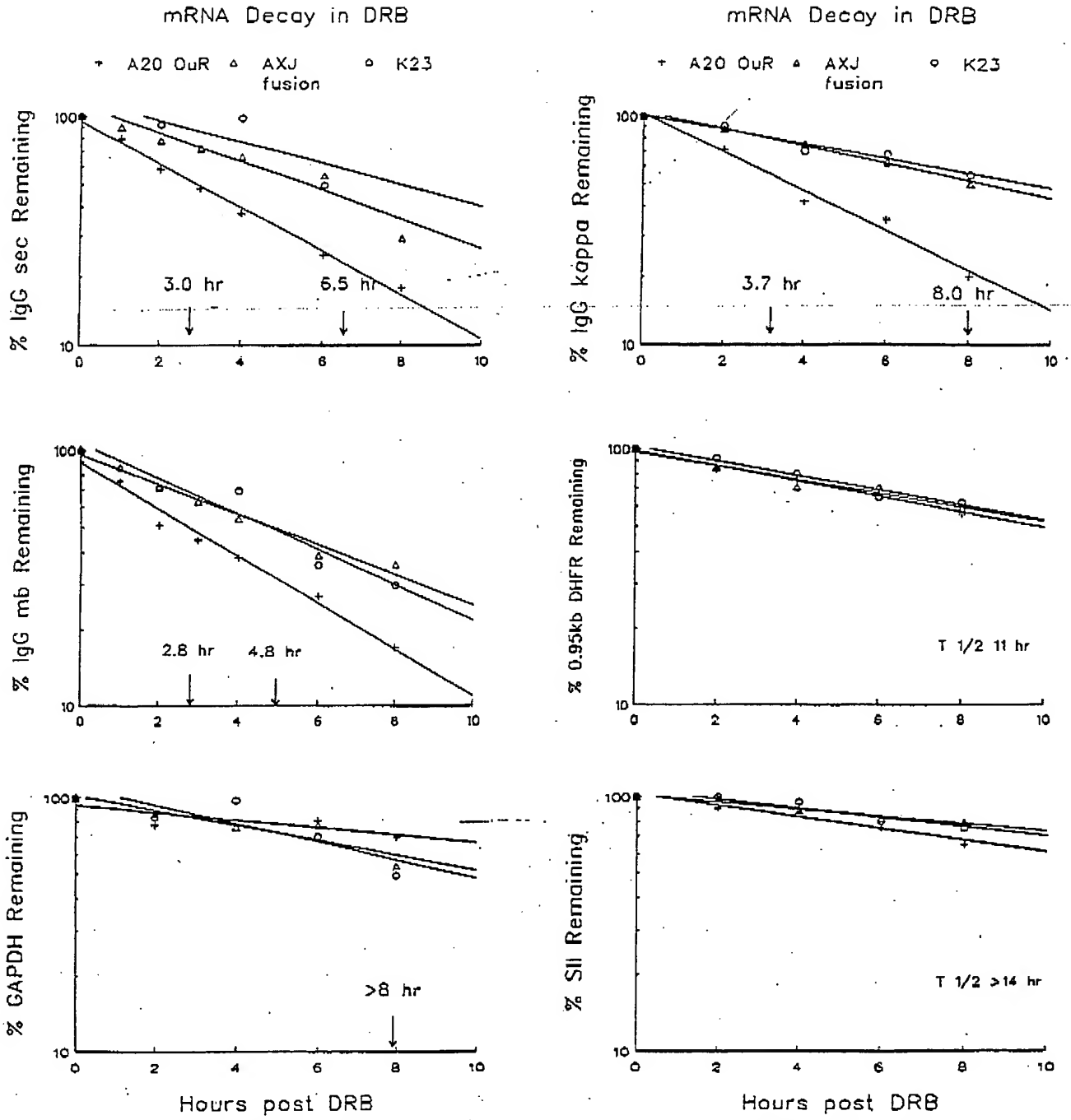

Fig. 6. Decay curves of various cytoplasmic mRNAs during DRB treatment. Data from Northern blots was used to determine the amount of a specific RNA remaining vs. time in DRB. Amounts of RNA were normalized to amount of 18S rRNA transferred to the nytran. Lines were generated from a least squares analysis. Error bars are not included for simplicity. +, A20.2J Ou^R B5; △, AXJ hybrid III; ○, K23 γ 2a myeloma. RNA half lives measured include: Ig γ 2a secretory (sec); Ig γ 2a membrane-specific (mem/mb); glyceroladehyde phosphate dehydrogenase (GAPDH); Ig κ light chain (Ig κ); dihyrofolate reductase, the 0.95 kb cytoplasmic species as shown in Fig. 5 (DHFR); transcription elongation factor SII (SII).

for the sec polyA site of the Ig γ gene (Lassman *et al.*, 1992; Milcarek and Hall, 1985) and the Ig μ and α genes (Peterson *et al.*, 1991; Seipelt and Peterson, 1995); the results presented here are in accord with those observations. The second effect is a decrease in the nuclear to cytoplasmic ratio for the sec γ 2a in the AXJ cells compared with the A20 parent with a smaller apparent change in the nuclear to cytoplasmic ratio for the γ 2a mem RNA. Note that the numbers obtained for the γ 2a membrane-encoding form are small and therefore difficult to quan-

Table 3. mRNA half-lives in A20 and hybrid cells

RNA:	Half-life value (in hr) A20	AXJ	J558L	K23
γ 2a sec	3.0±0.3	6.5±0.2	–	5.8±0.2
γ 2a mem	2.8±0.3	4.8±0.4	–	4.8±0.4
κ Light chain	3.7±0.3	8.0±0.7	–	9.0±0.8
DHFR	11	11	11	11
GAPDH	8	8	8	8
SII	14	14	14	14

Data from several experiments, including the one shown in Fig. 6, was used to determine the amount of a specific RNA remaining vs time in DRB. Amounts of RNA were normalized to amount of 18S rRNA transferred to the nytran. Lines were generated from a least squares analysis. Ig γ 2a secretory (sec); Ig γ 2a membrane-specific (mem); glyceroladehyde phosphate dehydrogenase (GAPDH); Ig κ light chain (Ig κ); dihydrofolate reductase, the 0.95 kb cytoplasmic species as shown in Fig. 5 (DHFR); transcription elongation factor SII (SII).

titate accurately. When we measured half-lives for the nuclear γ 2a sec and mem species in A20 vs AXJ cells we saw little difference between them (data not shown). We therefore believe that the change in nuclear to cytoplasmic ratio for the two lines reflects either an increase in the amount of sec γ 2a RNA leaving the nucleus in the AXJ hybrids or a retardation in the A20 cells. The change in the nuclear to cytoplasmic ratio for κ Ig light chain RNA parallels that seen for the Ig sec γ 2a heavy chain, indicating that there may be a coordinate regulation. When we looked at several genes we found that the AXJ hybrids retained less RNA in the nucleus than did the A20 cells, but Ig γ sec and κ light chain stand out as the ones that are most effected. In contrast, the Ig γ 2a mem RNA behaves like cyclophilin, GAPDH and SII by showing only a small change in distribution between A20 and the AXJ hybrids. In other experiments we show that transcription termination and mRNA half-life play little or no role in influencing the sec to mem ratios between A20 and the AXJ hybrid.

Factors contributing to changes in overall γ 2a cytoplasmic mRNA abundance

The absolute amounts of γ 2a RNA (sec + mem) in the nucleus of the A20 and AXJ hybrids differ very little. We see virtually no difference in the rates of transcription of the Ig γ 2a gene between A20 and the AXJ hybrid. The observed increase in post-transcriptional RNA processing at the Ig γ 2a sec polyA site might not be expected to influence the absolute amount of γ 2a sec + mem nuclear RNA significantly, because mature mRNAs are made from the Ig γ transcripts regardless of which polyA site is used. In contrast to the results obtained with nuclear RNA, the AXJ cells have 9.4-fold more Ig γ 2a sec RNA in the cytoplasm than do the A20 cells. The 2-fold increase in cytoplasmic half-life we observe for both the sec and mem γ 2a mRNAs contributes to the increase in overall γ 2a abundance in the AXJ hybrids. A larger contributor, however, to the increased cytoplasmic abundance of Ig γ2a mRNA is the change in the nuclear to cytoplasmic ratio for the two lines; this change reflects an increase in the overall amount of γ 2a RNA leaving the nucleus in the AXJ hybrids, most of it as the sec form. It is clear that recognition of some features of RNA, such as splicing or the absense of nonsense codons, occurs in the nucleus before export (Belgrader *et al.*, 1994); our work suggests, for the first time, that these recognition mechanisms may differ for at least some messages between B-cells in a differentiation stage-specific fashion. The mechanism of the alteration remains to be explored.

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